Shelf-Life Extension of Fresh Mushrooms (Agaricus bisporus) By Application of Hydrogen Peroxide and Browning Inhibitors

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ABSTRACT: An experimental washing process for fresh mushrooms entailing immersion in 5% H₂O₂, followed by application of a sodium crythorbate-based browning inhibitor, was optimized, scaled up, and made continuous. The laboratory process described previously was modified by adding a pre-wash step using 0.5% to 1% H,O,, increasing the wash solution H₂O₂ concentration from 3% to 5%, and substituting 4% sodium erythorbate + 0.1% NaCl for the more complex browning inhibitor formulation used previously. A continuous, commercial-scale washing facility was built to test the new process. Mushrooms washed by this process were free of adhering soil, less subject to brown blotch than conventionally washed mushrooms, and at least as resistant to enzymatic browning as unwashed mushrooms during storage at 4°C. Storage at 10°C accelerated development of brown blotch and browning.

Key Words: mushrooms, washing, hydrogen peroxide, browning inhibitors, shelf life

Introduction

THE SHELF LIFE OF FRESH MUSHROOMS MAY BE LIMITED BY L bacterial spoilage (Doores and others 1987) or enzymatic browning (Sapers and others 1994), depending on condition at harvest and on handling and storage conditions. Washing mushrooms to remove adhering compost or casing residues produces an attractive product for fresh market but may accelerate browning (Nichols 1985) or development of purple blotches (Sapers and others 1994). Washing also predisposes mushrooms to spoilage by Pseudomonas tolaasii and other bacteria as a consequence of mechanical injury to the mushroom surface and water absorption that results in high internal humidity (Beelman and others 1989; Sapers and others 1994). This defect is described as "brown" or "bacterial" blotch.

Hughes (1959) described a wash containing 0.05% sodium metabisulfite and 0.05% sodium chloride that could be used to clean mushrooms without aggravating browning. Treatment of mushrooms with sulfite, however, is no longer permitted (FDA 1986). Use of wash water containing hypochlorite was only partially effective in delaying spoilage (Wong and Preece 1985) and induced darkening at concentrations as low as 50 ppm (Sapers and others 1994). Guthrie and Beelman (1989) developed a wash containing stabilized chlorine dioxide, sodium erythorbate, and calcium chloride that was more effective than sulfite.

Hydrogen peroxide (H2O2) vapor has been used to sterilize medical supplies and equipment (Block 1991), aseptic packaging systems (Wang and Toledo 1986), and fresh table grapes (Forney and others 1991). Previously, we reported that such treatments were effective in suppressing lesion formation on mushrooms by P. tolaasi but induced browning during the 45 to 60 min exposure times required (Sapers and others 1995; Sapers and Simmons 1998). McConnell (1991) reported that a wash containing 1% H₂O₂ and 0.1% EDTA improved mushroom shelf life and reduced the incidence of discoloration during storage at 12 °C. In preliminary studies, we demonstrated that the shelf life of fresh mushrooms could be extended by application of a 3 % to 5% H₂O₂ wash followed by dipping in a browning inhibitor solution (Sapers

and others 1994, 1995). Such treatment was effective in reducing bacterial populations in wash water and on mushroom surfaces (Sapers and Simmons 1998) and had minimal effects on mushroom structure and composition (Sapers and others 1999). Commercial application of a H₂O₂/browning inhibitor treatment might enable producers to market washed mushrooms having superior quality and shelf life. Therefore, our objectives were to (1) optimize an integrated process for washing mushrooms with H2O2 as an anti-microbial treatment followed by application of a browning inhibitor solution; (2) scale up the washing process; and (3) evaluate the quality and shelf life of mushrooms washed by the experimental process under commercial-like conditions.

Materials and Methods

Raw materials

Fresh 1st- and 2nd-break (1st and 2nd crop from same bed) mushrooms (Agaricus bisporus) were obtained in tray packs from a grower in Kennett Square, Pa., U.S.A., chilled over ice, brought to the Eastern Regional Research Center (ERRC) within 2 h of harvest, and stored at 4 °C. Experiments performed at ERRC to evaluate the efficacy of H2O2 and browning inhibitor treatments were initiated within 24 h. Packing plant washing trials were carried out using facilities of a cooperating commercial mushroom packer in Avondale, Pa., U.S.A. Mushrooms used in these trials were designated as 1st quality or a lower grade suitable for canning (commercial or soup grade) by the packer. Bulk quantities of mushrooms in lug boxes containing about 4.5 kg, or an equivalent quantity of mushrooms packed in tills, were removed from 3 °C storage on pallets, as required.

Application of treatments at ERRC

Sets of 6 whole mushrooms were placed in the space between 2 nested colanders; immersed in 3% to 5% H₂O₂ for 30 s or 45 s; drained; and then dipped for 20 s in a browning inhibitor solution containing 2.25% or 4.5% sodium erythorbate and (1) 0.2% cysteine•HCl + 0.1% disodium EDTA, ad-

Table 1-Effect of H₂O₂ and browning inhibitor dips on discoloration of external mushroom surfaces during storage at 4 °C

		ı	_*-value				
H_2O_2	Erythorbate	Day			Appearance on Day 8		
Dip (%) ^a	Dip (%)	0	6	8	Lesions ^b	Browning ^b	
0	0	93.3°	90.8 ^{efg}	89.6 ^{efg}	++	+++	
3	2.25°	93.2 ^e	90.7 ^{fg}	89.8 ^{efg}	-	+	
_	2.25/NaCld	93.0e	93.1 ^{ef}	91.4ef	**	+	
	4.5°	93.0e	92.9ef	91.8ef	-	+	
	4.5/NaCld	92.4e	93.6°	92.1°	-	++	
5	2.25°	93.0°	90.4 ^{fg}	89.2 ^{fg}	•••	++	
-	4.5°	92.2e	88.79	87.5 ⁹	-	++	

a30 s dip.

bNone = -; slight = +; moderate = ++; severe = +++.

justed to pH 5.5 with 10% NaOH; or (2) 0.03-0.1% NaCl. Following treatment, the mushrooms were briefly drained and equilibrated in air at about 20 °C for 15 min before being packaged in 0.28-kg (10-oz.) plastic boxes (tills) and covered with punctured polyvinyl chloride film. In some experiments, treated mushrooms were cut in half along the stipe axis prior to application of browning inhibitors so that the effect of treatment on cut surfaces could be determined. Treated mushrooms and controls were stored at 4 °C or 10 to 12 °C.

Mushroom samples were evaluated initially and during storage by measurement of reflectance at the top of the pileus (whole mushrooms) or at the cut surface (halves) with a Byk-Gardner spectrocolorimeter (Sapers and others 1994). Samples were observed visually by 2 of the authors for severity of browning on the pileus top surface and sides, stipe circumferential surface, and bottom cut surface, and for severity of bacterial blotch on any external surface. Mushroom halves were examined visually for browning and lesion development on cut surfaces. Because of extensive mushroomto-mushroom variability in occurrence and severity of browning and lesions within samples (most mushrooms showing minimal defects), we made no attempt to quantify defect frequency. Since the presence of a single conspicuous defect in a sample container would render that sample unacceptable to consumers, we rated defects as "slight" to "severe," depending on the appearance of the worst mushrooms in the sample. In trials with replicated sample containers, we based the overall sample rating on the appearance of the worst container.

Scaled-up batch treatments

In trials conducted at a cooperating packing house, multiple batches of mushrooms weighing about 2.3 kg to 3.6 kg were placed in stainless steel baskets and covered with a perforated lid. The mushrooms were pre-washed to remove adhering soil by immersion for 20 s with agitation in 114 L of water, 0.5% or 1.0% H₂O₂ in a high-density polyethylene tank and were briefly drained. Each basket load of pre-washed mushrooms was immersed in the anti-microbial wash, $5\% H_2O_2$, for 30 s and then briefly drained. The washed mushrooms were treated with a browning inhibitor, 4% sodium erythorbate + 0.1% sodium chloride, by immersion in 151 L of solution for 20 s. The treated mushrooms were equilibrated for 30 min to 60 min at 3 °C, packaged in plastic tills, over-wrapped with punctured polyvinyl chloride film, and vacuum cooled for 30 min to lower their temperature and remove added water. Mushrooms were stored on-site at 3 °C and also brought

to ERRC for storage at 4 °C. Samples were evaluated as described above. The ERRC investigators and the plant-manager, who was experienced in recognizing commercially significant defects, jointly made observations at the packinghouse.

Continuous mushroom washing process

The batch pre-wash and anti-microbial wash treatments described above were made continuous by floating mushrooms through these solutions in stainless steel tanks, using conveyors to transfer mushrooms from 1 tank to the next and to permit drainage. Pre-wash solution was periodically discarded when the suspended soil level became excessive and the H₂O₂ concentration dropped below 0.5%. An antifoam agent was added to the 5% H₂O₂ wash solution. Immersion times were matched to those used in the batch process. Browning inhibitor solution was applied as a spray rather than by immersion. Subsequent steps were the same as used in the batch process. Samples were evaluated by visual examination by the ERRC investigators and packing plant manager without use of spectrophotometry, since the primary storage defects were darkening of the pileus sides and bacterial blotch development, neither of which could be measured reliably by reflectance.

Statistical analysis

Replicate L*-values, each measured at the pileus top surface of 8 whole mushrooms per treatment or at the cut surface of 8 mushroom halves (representing 4 mushrooms per treatment), were analyzed for treatment effects by analysis of variance with SAS/STAT software (SAS Institute Inc. 1989). Means were compared by the Bonferroni least significance difference mean separation test (Miller 1981).

Results and Discussion

Optimization of laboratory washing process

Since earlier studies demonstrated the potential of $\rm H_2O_2/ery$ thorbate dips in extending the shelf life of fresh mushrooms (Sapers and others 1994, 1995), we investigated the parameters of these treatments in greater detail to facilitate optimization and scale-up of the washing process. A comparison of whole mushrooms washed with 3% against 5% $\rm H_2O_2$, followed by dipping in 2.25% or 4.5% sodium erythorbate + 0.2% cysteine•HCl + 0.1% disodium EDTA indicated greater browning at the higher $\rm H_2O_2$ concentration (Table 1). Both browning inhibitor formulations and both erythorbate concentrations were effective in controlling browning. This

^{°20} s dip in 2.25% or 4.5% sodium erythorbate containing 0.2% cysteine • HCl + 0.1% disodium EDTA.

d20 s dip in 2.25% or 4.5% sodium erythorbate containing 0.1% NaCl.

e-9Means in the same column with no letter in common are significantly different (p < 0.05) by the Bonferroni LSD method.

able 2—Effect of H₂O₂ and browning inhibitor dips on disoloration of cut surfaces of mushroom halves during storge at 4 °C

		L*-val	ue					
	Day			Appearance on Day7 ^b				
reatmenta	0	5	7	Lesions	Browning	Graying		
ontrol	91.0°	89.9 ^d	87.0e	+	+	++		
l ₂ O ₂ + 2.25 laE	92.6°	96.3°	95.1°	+		+++		
I ₂ O ₂ + 2.25 IaE/NaCl	92.8°	95.0°	92.9 ^d	-	addr	++		
l ₂ O ₂ + 4.5 laE	91.8°	96.0°	95.1°	-	-	+++		
l ₂ O ₂ + 4.5 laE/NaCl	91.2°	96.1°	95.0°	-	-	++		

30 s dip in 5% $\rm H_2O_2$, drained, 20 s dip in 2.25% or 4.5% sodium erythorbate ontaining either 0.2% cysteine • HCl + 0.1% disodium EDTA (NaE treatment) r 0.1% NaCl (NaE/NaCl treatment).

None = -; slight = +; moderate = ++; severe = +++. NeMeans in the same column with no letter in common are significantly different (p < 0.05) by ne Bonferroni LSD method.

was more evident in the visual observations of browning on he pileus sides and stem than on the pileus top surface, where the somewhat inconclusive spectrocolorimetric data were obtained. The sodium erythorbate/NaCl formulation is nore economical since NaCl is less expensive than cysteine•HCl or disodium EDTA. Both formulations were effective in controlling browning of mushroom halves in which the cut surface represented the response of sliced mushrooms (Table 2). In these trials, the spectrocolorimetric measurement of "L" was a good indicator of browning inhibition. However, the sodium erythorbate/NaCl formulation appeared to suppress graying of the cut surface during storage, a defect that appeared to result from the senescence of gill tissue and diffusion of gill pigments into the adjacent pileus flesh.

Mushroom halves washed with 3% H₂O₂ showed no lesion development on cut surfaces during storage for 7 d. With whole mushrooms, no consistent difference in the number or severity of bacterial lesions could be seen between samples treated with 3% or 5% H₂O₂ and stored for 7 d to 8 d at 4 °C. However, some visual evidence of improved quality was seen in samples that were washed with 5% H₂O₂ for 30 s, dipped in the sodium erythorbate/NaCl solution, and then stored for as long as 10 d (Table 3). Washing in H₂O₂ for 45 s had a detrimental effect, resulting in more lesion development and browning, probably because of greater water uptake. In subsequent washing experiments, the higher H₂O₂ concentration was selected with the expectation that it might be more effective with mushrooms having higher bacterial loads and/or containing more adhering soil.

Scaled-up batch washing process

When the laboratory-scale washing process was relocated to a mushroom packing house location and scaled up from about 100-g to 50-kg quantities, it became evident that soil adhering to freshly harvested mushrooms would rapidly accumulate in the 5% H₂O₂ wash solution and limit its life. Therefore, we introduced a pre-wash step in which the stainless steel baskets of dirty mushrooms were immersed in water to remove most of the adhering soil before washing in H₂O₂ solution. This step had no consistent effect on the quality or shelf life of good quality raw materials irrespective of mushroom break (Table 4), although in some trials, prewashing with water appeared to destabilize mushrooms (data not shown). This may have been due to excessive water

Table 3—Effect of H₂O₂ concentration and treatment time on storage stability of whole mushrooms at 4 °C

H ₂ O ₂ treatment		L*-value		Lesions ^b Day		Browning ^b Day		
Concn Time		Day						
(%)	(S)	0	8	10	8	10	8	10
0	0	93.8°	90.9 ^d	89.8 ^d	++	++	+++	+++
3	30	89.79	92.1 ^{cd}	91.4 ^{cd}	-	-	++	++
_	45	91.5 ^d	92.7 ^{cd}	90.8 ^d	-	++	+	+++
5	30	91.8 ^d	93.9°	92.7°	-	-	+	++
-	45	91.3 ^d	92.7cd	91.5 ^{cd}	+	++	++	+++

 $^{\rm a}{\rm H_2O_2}$ treatment followed by 20 s dip in 4.5% sodium erythorbate + 0.1% NaCl.

PNone = -; slight = +; moderate = ++; severe = +++.

cdeMeans in the same column with no letter in common are significantly different (p < 0.05) by the Bonferroni LSD method.

uptake, which would increase the internal humidity and favor growth of spoilage organisms (Beelman and others 1989). Typically, pre-washing resulted in an increase in weight of about 3% to 4%. Vacuum cooling is an optional treatment applied to packaged fresh mushrooms prior to refrigerated storage and shipping. In some trials, vacuum cooling appeared to increase lesion formation and browning in the washed samples. However, in most of the trials, vacuum cooling had no adverse effects on quality. The variable response of washed mushrooms to vacuum cooling may be related to the time interval between washing and loading into the vacuum chamber (in some cases as much as several hours) during which samples were not refrigerated.

Continuous washing process

During the transition from batch to continuous washing, mushrooms given alternative pre-washing treatments, applied by dipping batch-wise, and mushrooms washed with 5% H₂O₂ by dipping or with the continuous system were compared. Reflectance data were not obtained for these samples since the most serious defects were bacterial lesions and browning on the pileus sides and stipe, which could not be measured reliably with the spectrocolorimeter. Pre-washing 1st-quality mushrooms with 0.5% H₂O₂ resulted in fewer lesions and less browning after storage at 3 °C for 7 d than pre-washing with water (Table 5). Differences were small between continuously washed and batch washed samples. However, mushrooms given the batch wash with 5% H₂O₂ but no pre-wash showed less lesion formation than continuously washed mushrooms. Lower grade commercial quality mushrooms did not respond well to the continuous or batch washing process, showing little or no improvement in quality after 7 d at 3 °C. Excessive foam in the 5% H_2O_2 tank, due to oxygen evolution from the catalase-catalyzed decomposition of H₂O₂, and the short residence time of mushrooms (about 13 s) in this tank (subsequently corrected) may have reduced the effectiveness of washing in these trials. Laboratory tests demonstrated that foam produced in the H2O2 wash could be completely suppressed by addition of either FG10 (Dow Corning Corp., Midland, Mich., U.S.A.) or SBI Defoamer 95601 (Systems Bio-Industries Inc., Waukesha, Wis., U.S.A.), both food-grade anti-foam agents, at the 5 ppm (active ingredient) level. The latter product was used successfully in subsequent continuous washing trials.

In the final series of trials with the continuous washing system, the pre-wash treatment was made continuous by inserting a pre-wash tank and conveyor to the 5% H2O2 wash tank at the beginning of the washing line, and the pre-wash

Table 4-Effect of pre-wash and vacuum cooling on shelf-life of mushrooms washed with 5% H₂O₂ and browning inhibitor solution

			L*-valu	e		
Raw	The state of the s	ingen en gegen gigt af de la general de l'Article de l'Ar	Day		Appearance of	on Day 7/8 ^b
Material	Treatment ^a	0	5	7/8 ^b	Lesions ^c	Browning ^c
1 st break	Control	92.1 ^{de}	88.2 ^f	87.4°		+
	PW only	90.6°	89.4 ^{ef}	86.4 ^e	+-+	+
	$H_2O_2 + BI$	92.3 ^{de}	93.0 ^d	90.9 ^d		+
	$H_2O_2 + BI + VC$	92.8 ^d	92.0 ^{de}	86.8°	++	+++
	PW + H ₂ O ₂ + BI	91.9 ^{de}	92.6 ^d	91.6 ^d		+
	$PW + H_2O_2 + BI + VC$	92.7 ^d	91.0 ^{de}	78.8 ^f	+++	+++
2 nd break	Control	92.1d	88.8 ^d	84.4 ^d	++	++
	PW only	91.9 ^d	82.8e	***	+++	+++
	H ₂ O ₂ + BI	92.0 ^d	90.5 ^d	87.0 ^d	+	++
	PW + H ₂ O ₂ + BI	91.8 ^d	90.7 ^d	86.9 ^d		+

PW = PPW =measured on D 8 because of severe lesions.

CNone = -; slight = +; moderate = ++; severe = +++

defFor each break, means in the same column with no letter in common are significantly different (p < 0.05) by the Bonferroni LSD method.

Table 5-Enhancement of quality of continuously or batchwashed mushrooms by addition of H₂O₂ to pre-wash water

Mushroom grade	5% H ₂ O ₂ wash treatment	Pre-wash treatment ^b	Appearance on Day 7°		
			Lesions	Browning	
1st quality	Control	None	+++	+++	
	Continuous	None	++		
		Water	+	+	
		0.5%H ₂ O ₂		-	
	Batch	None	-	-	
		Water	+	+	
		0.5% H ₂ O ₂	+	NAME.	
Commercial	Control	None	+++	+++	
	Continuous	None	+++	+++	
		Water	+++	++	
		0.5% H ₂ O ₂	+++	+++	
	Batch	None	+++	+++	
		Water	++	+++	
		0.5% H ₂ O ₂	++	++	

 $^{\rm a}$ Mushrooms conveyed through tank containing 5% $\rm H_2O_2$ for about 13 s or dipped batch-wise for 30 s in 5% $\rm H_2O_2$, drained and sprayed with 4% sodium erythorbate + 0.1% NaCl + 0.1% CaCl₂.

bBatch pre-wash applied as 20 s immersion of mushrooms contained in

cAt 3 °C. None = -; slight = +; moderate = ++; severe = +++.

 $\rm H_2O_2$ concentration was increased to 1%. In addition, 0.1% CaCl₂ was added to the browning inhibitor solution, and conveyor speeds were adjusted to provide residence times of 20 and 30 s for the pre-wash and 5% H₂O₂ treatments, respectively. The effects of these modifications on product quality were determined in 2 series of trials in which portions of 1st-quality mushrooms weighing about 18 kg were alternated with portions of lower grade "soup" mushrooms weighing about 180 kg until a total of about 839 kg (730 kg lower grade + 109 kg 1st-quality mushrooms in Series I) or about 617 kg (544 kg lower grade + 73 kg 1st-quality mushrooms in Series II) had been washed (Table 6). By alternating the 2 grades in this manner, we could determine the capacity of the washing solutions and effect of throughput on product quality using a large mass of expendable raw material rather than the more valuable 1st-quality mushrooms. The trials were run approximately 1 y apart with raw materials that were representative of commercial practice. By this approach we could determine whether the accumulation of soil, reactive substances extracted there from, or microor-

the washed 1st-quality mushrooms over an extended production run (that is, 2 h to 3 h). Mushroom samples were examined after storage at 4 and 10 °C, the latter temperature representing storage abuse during marketing.

These experiments showed that mushrooms given the continuous washing treatment were superior to unwashed or washed controls with respect to both lesion suppression and control of browning during storage. The Series II unwashed control mushrooms were of poorer quality than the Series I controls, showing browning and ginger blotch development, a defect attributed to a Pseudomonas fluorescens strain different from P. tolaasii (Wong and others 1982, Wells and others 1996). Nevertheless, the hydrogen peroxide washing process was able to control these defects in the Series II trials. Extended use of the washing system had little or no effect on the quality of mushrooms stored at 4 °C. However, at 10 °C, samples showed more lesion development but little change in browning as the total quantity of mushrooms processed increased. This may be an indication of bacterial build-up in the pre-wash tank, in the browning inhibitor solution reservoir, and on conveyors, since bacterial survival in 5% H₂O₂ contained in the wash tank should have been minimal (Sapers and Simmons 1998). The concentration of H₂O₂ did not change appreciably during extended trials. The erythorbate concentration gradually decreased during extended operation of the washing line, presumably due to oxidation. Maintenance of the sodium erythorbate concentration in the browning inhibitor solution between 3% and 4% should be sufficient to prevent sample browning during storage. Increased attention to sanitation during raw material handling, washing, and packaging and avoidance of temperature abuse during marketing are recommended in order to maintain mushroom quality during extended storage.

The benefits of the continuous hydrogen peroxide washing process with mushrooms may be applicable to other fresh fruits and vegetables where microbiological issues limit quality or food safety. Research on hydrogen peroxide washing of apples, melons, and other commodities is under way in our laboratory.

Conclusions

TIRST-QUALITY MUSHROOMS WASHED WITH DILUTE H_2O_2 SO-I lutions and sprayed with a sodium erythorbate-based browning inhibitor solution in an experimental continuous ganisms in the system could affect the quality or shelf life of washing line were less subject to brown blotch lesions than

Table 6-Effect of quantity of mushrooms washed in continuous system on product quality*

		Day 7	at 4 °C	Day	5 at 10 °C	
Series	Treatment of 1st-quality mushrooms	Lesions	Browning	Lesions ^c	Browning ^c	
I	Control	***	+	_	++	
	Before soup grade ^b	-	1664	+		
	After 186 kg soup grade ^b				_	
	After 367 kg soup grade ^b		-	+++		
	After 549 kg soup gradeb,d	+	-	+++	+	
	After 730 kg soup grade ^b			+	_	
11	Control	+++	-	++	+	
	Conventional wash	+++	+++	+++	+++	
	Before soup grade ^b	-	+	-	+	
	After 181 kg soup grade ^b	-	•••	-	+	
	After 363 kg soup grade ^b			++	+	
	After 544 kg soup grade ^b	ware	+	+++	++	

 4 For each trial, 18 kg 1 SL-quality mushrooms pre-washed in 1% 4 H $_{2}$ O $_{2}$ for 20 s, washed in 5% 4 H $_{2}$ O $_{2}$ for 30 s, drained, and sprayed with 4% sodium erythorbate + 0.1% NaCl + 0.1% CaCl $_{2}$ in continuous washing system; then equilibrated in air, packaged and vacuum cooled.

conventionally washed mushrooms and showed no more enzymatic browning than was observed on unwashed controls.

With proper management of wash solution strength, the quantity of mushrooms washed in the system had a minimal effect on product stability during storage at 4 °C.

The experimental washing process did not improve the quality and stability of low-grade mushrooms. Storage at 10 °C of mushrooms given the experimental washing process, an abusive condition, accelerated lesion development and enzymatic browning.

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None = -; slight = +; moderate = ++; severe = +++.

dPre-wash solution replaced.